

REMARKS

Claims 1-19 were pending before the Office. Claims 1, 3, 4 and 10 have been amended. Claims 15, 16 and 18 have been cancelled. New claim 20 has been added. Accordingly, claims 1-14, 17 and 19-20 shall be pending upon entry of this amendment.

The amendments made herein have been made solely to claim more fully the invention and/or to expedite prosecution of the present application and should in no way be construed as an acquiescence to any of the rejections in the Office Action issued in the present application. Applicants reserve the right to pursue the subject matter of the claims as originally filed or similar claims in one or more subsequent applications.

Support for the amendments can be found throughout the originally-filed application, including the specification, examples and claims.

For example, support for the amendment to claim 1, which now specifies step (g) of “renaturing” the IL-4 or muteins thereof can be found, for instance, on page 13, lines 17-20 of the published corresponding PCT application, WO 2004/007549.

Support for new claim 20 can be found throughout the application and original claims as-filed, for example, at page 12, line 10 to page 14, line 26.

No new matter has been added by this amendment.

The rejections under 35 USC §112, 2nd paragraph, are overcome

The Office Action rejects claim 3 under 35 U.S.C. 112, second paragraph, as allegedly being indefinite. Applicants, while not intending to acquiesce as to the rejection, have amended claim 3 to clarify that the washing buffer comprises the claimed non-ionic detergent, ionic surfactant or zwitterionic detergent. Support for this amendment can be found, for example, on

page 8, lines 20-26, of the published corresponding PCT application, WO 2004/007549.

The Office Action rejects claim 10 under 35 U.S.C. 112, second paragraph, as allegedly being indefinite. Applicants, while not intending to acquiesce as to the rejection, have amended claim 10 to clarify that the dependency should be from claim 9, rather than from claim 1.

Accordingly, Applicants respectfully request reconsideration and withdrawal of these Section 112 rejections.

The rejections under 35 USC §103(a) are overcome

The Office Action rejects claims 1-6, 8, 11, 13, 14, 17 and 19 under 35 U.S.C. §103(a) as being unpatentable over Domingues et al. (Journal of Biotechnology, “Improving the refolding yield of interleukin-4 through the optimization of local interactions,” 2000, 84:217-230) (herein as “DOMINGUES”) in view of Wyllie et al. (U.S. Patent No. 5,932,102) (herein as “WYLLIE”). The Office Action concludes that all of the claimed steps of independent method claim 1 (on which all other claims ultimately depend) are taught by DOMINGUES except for steps (e) and (f) which pertain to separating the denatured IL-4 (or its muteins) using an IMAC (immobilized metal affinity chromatography) system and releasing and refolding same, respectively. More in particular, the Office Action states that “Wyllie et al. teach a method for purifying a protein containing histidine residues using immobilized metal affinity chromatography (Abstract). Wyllie et al. teach that human IL-4 and 5 histidine residues and is predicted to have high affinity to the immobilized metal.” The Office Action concludes that “it would have been obvious to one having ordinary skill in the art...to modify the method Domingues et al. to purify the denatured IL-4 or muteins thereof using an immobilized metal chelate affinity chromatography with a reasonable expectation of success” and that “[o]ne would have been motivated to do so because

an immobilized metal chelate affinity chromatography provides an alternative approach for purifying IL-4.” See Office Action, page 4.

In addition, the Office Action rejects the following dependent claims as being unpatentable in view of DOMINGUES and WYLLIE and in further view of one or more references, as follows.

Claim 7 is rejected under 35 U.S.C. 103(a) as being unpatentable over DOMINGUES and WYLLIE as applied above, and in further view of Apeler et al. (EP 1022337 A2). The Office Action states that Apeler et al. teaches the expression of the particular human interleukin-4 mutant, R121D Y124D, that is claimed.

Claims 9 and 10 are rejected under 35 U.S.C 103(a) as being unpatentable over DOMINGUES and WYLLIE as applied above, and in further view of “Apelar et al.” but then cites to “Gellman et al.” as teaching the use of an artificial chaperone, such as beta-cyclodextrin. The Gellman et al. reference is not identified in the Office Action and thus, Applicants have not had the opportunity to fully consider this rejection. Applicants respectfully request the Examiner to identify Gellman et al.

Claim 12 is rejected under 35 U.S.C. 103(a) as being unpatentable over DOMINGUES and WYLLIE as applied above, and in further view of Bonsch et al. (J. Biol. Chem., 270:8452-8457). The Office Action states that Bonsch et al. teaches the expression of the particular murine homolog of human interleukin-4 that is claimed, Q116D and Y119D.

Claim 15 and 16 are rejected under 35 U.S.C. 103(a) as being unpatentable over DOMINGUES and WYLLIE as applied above, and in further view of Thogersen et al. (U.S. Patent No. 5,739,281). The Office Action states that it would have been obvious to carry out the

claimed method with the refolding step being performed while the IL-4 remains bound to the IMAC system in view of the teachings in Thogersen et al. pertaining to the use of matrix-assisted folding of various polypeptides.

Applicants respectfully disagree with the Section 103 rejections and traverse as follows.

As the Office will appreciate, Graham v. John Deere Co., 338 U.S. 1, 148 USPQ 459 (1966), was reaffirmed by KSR International Co. v. Teleflex Inc., 127 S.Ct. 1727, 82 USPQ2d 1385 (2007) as providing the correct analytical framework for determining obviousness. Under Graham, obviousness is a question of law based on underlying factual inquires that address (1) the scope and content of the prior art, (2) the differences between the claimed invention and the prior art, and (3) the level of ordinary skill in the pertinent art. *Additionally*, the Supreme Court in KSR required a “clear articulation of the reason(s) why the claimed invention would have been obvious” and that such reason “supporting a rejection under 35 U.S.C. 103 should be made explicit.” Here, the rationale used to support the rejection was that there was “some teaching, suggestion or motivation in the prior art references that would have led one of ordinary skill in the art to modify the prior art reference or to combine prior art reference teachings to arrive at the claimed invention.” M.P.E.P. 2143 (G). This rationale requires the Office to articulate (1) a *teaching, suggestion or motivation* to combine the references and (2) a finding of a *reasonable expectation of success*. Applicants respectfully submit that the obviousness rejection is not supported by either a teaching, suggestion or motivation to combine or a reasonable expectation of success, and thus, should be withdrawn.

The Office states that DOMINGUES teaches all but two of the claimed steps of claim 1. In particular, the Office Action indicates that DOMINGUES teaches “a method for purifying

interleukin-4 or mutants by recombinant expression comprising (a) expression in inclusion bodies...(b) disrupting the cells and separating the inclusion bodies, (c) washing inclusion bodies obtained with 0.1 M Tris-HCl pH 8/1 mM EDTA/0.1% zwittergent, (d) solubilizing (sp) the inclusion bodies in 8 M GdnHCl, (e) renaturing the expression product and purifying the expression product by cross-flow ultrafiltration against five volumes of buffer.” According to the Office Action, DOMINGUES fails to teach steps (e) and (f) of claim 1, which pertain to the separating of the denatured IL-4 or muteins thereof using an immobilized metal chelate affinity chromatography (IMAC) system, followed by their release from the column and then their refolding.

Turning to WYLLIE, the Office Action states that this reference teaches a method for purifying proteins using IMAC and that the IL-4 is predicted to have high affinity to the IMAC resin. The Office Action concludes that it would have been obvious to modify DOMINGUES with the additional steps of WYLLIE to reach the claimed invention because there was both a motivation to make the combination (IMAC being an alternate approach to purifying IL-4) and a reasonable expectation of success (because IL-4 has 5 histidine residues and is alleged to have high affinity to the IMAC resin). Applicants strongly disagree with this rejection.

Applicants respectfully submit that WYLLIE is mischaracterized and that one of skill in the art would not have been motivated to combine WYLLIE and DOMINGUES nor would such a person have had any reasonable expectation of success in doing so.

The Office is respectfully reminded that “***All words in a claim must be considered in judging the patentability of that claim against the prior art.***” M.P.E.P. 2143.03, citing *In re Wilson*, 424 F.2d 1382, 1385 (CCPA 1970). Here, claim 1 is directed to a method of purifying

IL-4 or muteins thereof that includes: step (d) *denaturing* the IL-4; step (e) separating the *denatured* IL-4 or muteins thereof using an IMAC system; step (f) releasing the IL-4 or muteins thereof from the IMAC system; and step (g) *renaturing* the IL-4 or mutiens thereof, thereby obtaining the purified IL-4. Thus the IL-4 that is applied to and subject to separation by the IMAC sytems and subsequent renaturation is the *denatured* form of IL-4.

Conversely, WYLLIE does not teach, suggest or even mention denatured IL-4 or denatured proteins generally. Instead, WYLLIE is concerned with the purification of *fully natured, native* proteins from crude cell extract that have not been treated with any denaturing agent. For instance, WYLLIE teaches at column 5 the purification of IL-4 from “a crude *E. coli* broth.” Similarly, WYLLIE teaches at column 7 the purification of IL-10 from a “a crude preparation from a murine melanoma cell culture” and the purification of gamma-interferon from a “crude *E. coli* fermentation broth.” *At no point does WYLLIE teach or suggest denaturing IL-4 or any protein, separating denatured IL-4 or any protein on an IMAC system, or renaturing IL-4 or any protein subsequent to IMAC separation and release.* Thus, WYLLIE relates particularly to an approach for determining the suitability of various proteins *in their non-denatured state* as candidates for purification by IMAC and the optimal conditions for such purification. It is respectfully submitted that to regard WYLLIE as a teaching of a method for predicting affinity of denatured proteins would be to mischaracterize the reference.

Not only does WYLLIE not pertain to denatured proteins, unlike the claimed invention, one of ordinary skill in the art would not have been motivated to combine WYLLIE with DOMINGUES. In particular, the skilled person would not have been motivated to combine WYLLIE together with DOMINGUES because WYLLIE *teaches directly away* from their

combination. The M.P.E.P. instructs that “It is improper to combine references where the references teach away from their combination. M.P.E.P. 2145(X)(D). Here, WYLLIE teaches that “IMAC should not be considered as a primary purification step for a protein predicted to possess a low affinity to the metal-chelating resins.” WYLLIE goes on to teach that “Without prediction of protein-resin affinity, purification development of IMAC may become an unnecessarily time consuming effort which may not yield useful results.” See column 1, lines 42-53. Applicants again submit that WYLLIE neither teaches or suggests anything about the affinity characteristics of the *denatured* form of human interleukin-4, i.e., the claimed form of IL-4. While WYLLIE, admittedly, discloses IMAC purification of *native* human IL-4, such knowledge is already admitted as being known in the prior art by Applicants at page 12 of the corresponding PCT application, PCT/EP2003/007022 (WO 2004/007549) at lines 30-31, which states that “it was known from the literature that native Interleukin-4 is bound to IMAC.”

Since WYLLIE does not teach or suggest the affinity characteristics of the denatured form of human IL-4, one of ordinary skill in the art would have no understanding, based on WYLLIE, of the affinity characteristics of the denatured form of human IL-4 to IMAC resins, and thus, would not be swayed to use the IMAC system to purify IL-4 in order to avoid an “unnecessarily time consuming effort which may not yield useful results.” That is, by WYLLIE’s own instruction, the skilled artisan should not use the IMAC system to purify a protein of interest unless there is knowledge as to the affinity characteristics of that protein to the IMAC resin. Since WYLLIE does not teach or suggest the requisite affinity characteristics of denatured IL-4, the skilled person would not turn to WYLLIE as an alternate approach for purifying the denatured human IL-4 as claimed.

Moreover, the skilled person would ***not*** have had a reasonable expectation of success as to reaching the claimed invention by the combination of DOMINGUES and WYLLIE because WYLLIE's teachings as to the suitability of a protein as an IMAC candidate are directed to native proteins only. Because the physical-chemical properties between native and denatured proteins are inherently different (the physical-chemical properties of a denatured protein are more a function of the linear amino acid sequence, whereas the physical-chemical properties of a native protein are more a function of both the linear amino acid sequence and the tertiary and/or quaternary structure), and because WYLLIE provides no guidance in determining how to predict the affinity of denatured proteins, WYLLIE would not provide the skilled person with any reasonable expectation of success in carrying out the invention. Indeed, the only information WYLLIE provides about human IL-4 that would also pertain to the denatured form of IL-4 is that the number of histidines present in the linear sequence is five (5). ***However***, WYLLIE specifically teaches that "Histidine availability, however, ***is not simply proportional to the total number of His residues in a protein***" and that "***the total number of His residues does not solely determine affinity to IMAC.***" See column 2, lines 9-10 and 35-36.

Still further, WYLLIE's method would not even be applicable to the denatured IL-4 of the claimed invention since WYLLIE's method is based on correlating binding-affinity data generated using ***native*** proteins against certain physical ***primary and tertiary*** structural characteristics of the tested proteins. More in particular, WYLLIE's method correlates certain ***tertiary structure information*** (e.g., solvent-exposed surface area – SESA) and ***primary sequence information*** (e.g., the hydrophilicity indices (HI) of each histidine in a protein) of a protein of interest against the relative affinity of that protein of interest for IMAC resin.

Because the correlation tested in WYLLIE was between certain primary and tertiary structural characteristics against measured binding affinities of *native* proteins, rather than *denatured* proteins, and because of the inherent differences in the physical-chemical properties of native and denatured proteins mentioned above, WYLLIE's method is *not* a suitable tool of prediction for the affinity of denatured proteins for IMAC resins. Given the above, and given that the field of protein purification is well-documented as being a highly-unpredictable field (e.g., "The problems associated with expressing and purifying human proteins, especially in *Escherichia coli*, the primary host organism for high-throughput (HTP) applications, are well-documented and have plagued researchers for decades." Basic Science Techniques in Clinical Practice, Chapter 9, Abstract, Eds. Patel, Arya and Shergill, Springer, 2007), ***one of ordinary skill in the art would not have had any reasonable expectation of success in view of WYLLIE to achieve the present invention.***

Thus, the Office's conclusion that "it would have been obvious to one having ordinary skill in the art...to modify the method Domingues et al. to purify the denatured IL-4 or muteins thereof using an immobilized metal chelate affinity chromatography with a reasonable expectation of success" and that "[o]ne would have been motivated to do so because an immobilized metal chelate affinity chromatography provides an alternative approach for purifying IL-4" is incorrect for at least the above reasons.

As to the remaining claims under rejection, "If an independent claim is nonobvious under 35 U.S.C. 103, then any claim depending therefrom is nonobvious." *In re Fine*, 837 F.2d 1071 (Fed. Cir. 1988). Each of the remaining claims under rejection, i.e., claims 2-14, 17 and 19, each

ultimately depend from claim 1. Since, as explained above, claim 1 is nonobvious, so also must be claims 2-14, 17 and 19.

In view of at least the above reasons, Applicants respectfully request reconsideration and withdrawal of the rejections under 35 U.S.C. 103.

CONCLUSION

In view of the remarks herein, Applicants respectfully request reconsideration and withdrawal of all of the rejections as Applicants believe the application to be in condition for allowance. Favorable reconsideration of the application and prompt issuance of a Notice of Allowance are respectfully requested. Please charge any required fee or credit any overpayment to Deposit Account No. 04-1105.

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Respectfully submitted,

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